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Higher N₂O emission by intensified crop production in South AsiaNani Raut^{a,*}, Bishal K. Sitaula^b, Lars R. Bakken^c, Roshan M. Bajracharya^a, Peter Dörsch^c^a Department of Environmental Science and Engineering, Kathmandu University, PO Box 6250, Dhulikhel, Nepal^b Department of International Environment and Development Studies (Noragric), Norwegian University of Life Sciences, 1432 Ås, Norway^c Department of Environmental Sciences, Norwegian University of Life Sciences, 1432 Ås, Norway

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ABSTRACT

Intensification of food production in Nepal has been found to acidify the soils and hence increase their apparent propensity to emit N₂O as measured by the N₂O/(N₂ + N₂O) product ratio of denitrification during standardized anoxic incubations (Raut et al., 2012). We hypothesized that this would lead to high N₂O emission factors (EF), and tested this by measuring N₂O emissions from fields on which intensified crop production (IC) had been practiced for the last 20 years, and adjacent fields having traditional crop production (TC) practices. The measurements were done every one to two weeks over a period of 12 months covering two to three cropping periods. On the sites with periodically flooded soils, the cumulated emissions for IC and TC were 15.41 and 7.23 kg N₂O/ha, respectively. On the sites with permanently drained soils, the cumulated emissions were 5.43 and 1.46 kg N₂O/ha (IC and TC). We used the available data on fertilizer levels to calculate an emission factor for the transition from TC to IC (EF_I); i.e. $\Delta N_2O - N / \Delta N_{fertilizer}$, where $\Delta N_2O - N$ is the cumulated emission in IC minus that in TC, $\Delta N_{fertilizer}$ is the annual N input to IC minus that in TC. The EF values were 0.08 and 0.02 for the sites with permanently drained and periodically flooded soils, respectively. These factors are 2 to 8 times higher than the EF values used by IPCC to calculate emission as a function of fertilizer level. The high EF_I appear to confirm our hypothesis that intensification will lead to higher emission of N₂O than that predicted by the increase in nitrogen inputs, and that this is due to the soil acidification.

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1. Introduction

Nitrous oxide (N₂O), a colorless, non-toxic and stable naturally occurring gas (Lassey and Harvey, 2007) is a stratospheric ozone depleting substance (Ravishankara et al., 2009). Anthropogenic activities have increased the availability of fixed nitrogen (N) in the biosphere (Beaulieu et al., 2011). The invention of the Haber–Bosch process gave rise to the introduction of synthetic nitrogen-based fertilizers the availability of which has enabled the expansion of intensive farming (Thomson et al., 2012). Thus greater N availability is leading to increasing emissions of N₂O. N₂O has an atmospheric lifetime of 114 years and 296 times stronger specific global warming potential than an equal mass of CO₂ (IPCC, 2007). Approximately 62% of the atmospheric N₂O is from natural and agricultural soils (Skiba and Smith, 2000; Smith et al., 2008).

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Table 1
Major cropping patterns in the Ansikhola watershed.

Cultivated land types	Agricultural system	
	Intensified	Traditional
Khet	Rice–potato–rice	Rice–rice
	Rice–potato–maize/rice–maize–rice	Rice–maize
	Rice–potato–maize	Rice–maize
	Rice–potato–vegetables	
	Rice–potato–tomato	
Bari	Vegetable–maize–potato/maize–mustard–potato	Maize–potato
	Chilly–vegetable–potato	Maize–millet
		Maize–millet/maize–wheat

Table 2

Cropping system, fertilizer practice, bulk density ($n = 2$), soil pH ($n = 4$) and soil texture ($n = 4$) for traditional and intensified cultivated sites for field flux measurements.

Cultivated land type	Agricultural system	Cropping pattern	DAP ^a (kg N ha ⁻¹)	Urea (kg N ha ⁻¹)	Compost (kg ha ⁻¹)	Bulk density ^c (g cm ⁻³)	Soil pH ^d	Texture ^b
Khet	Traditional	Rice–Rice	40	120	12,279	1.19	4.94	Clay loam
	Intensified	Rice–maize–rice	94	210	16,372	1.26	4.27	Clay loam
Bari	Traditional	Wheat–maize	52	143	21,499	1.30	5.05	Sandy loam
	Intensified	Maize–potato–mustard	75	184	24,558	1.14	4.21	Sandy loam

^a DAP = di ammonium phosphate.

^b Soil texture classification was done according to the USDA system ($n = 4$).

^c Mean soil bulk density ($n = 2$).

^d Mean soil pH ($n = 4$) measured in 0.01M KCl.

In many south Asian countries, intensified cropping systems are replacing the subsistence-based traditional farming system in order to meet the increasing demand for food production (Rasul and Thapa, 2003; Paudel and Thapa, 2004; Brown and Kennedy, 2005; Tiwari et al., 2008). The traditional farming was characterized by low fertilizer levels and a single crop or two crops per year. Intensified systems have higher inorganic fertilizer levels and a minimum of three crops per year.

N₂O emission is the by-product of nitrification and denitrification. Denitrification refers to the step-wise reduction of nitrate or nitrite to gaseous products, i.e., NO, N₂O and N₂. The study of denitrification kinetics and its product stoichiometry (NO/N₂O/N₂) under standardized laboratory incubations has been used to characterize the propensity of soils to emit NO and N₂O to the atmospheres (Raut et al., 2012; Qu et al., 2014; Jones et al., 2014). In theory, the propensity to emit NO and N₂O depends on the composition of the microbial community, because the regulatory phenotypes of denitrifying organisms are variable (Bakken et al., 2012), and circumstantial evidence for a role of gene abundance has been provided (Jones et al., 2014). However, the product ratio of denitrification is also strongly affected by soil pH, and the reason appears to be that the expression of N₂O reductase is increasingly difficult with decreasing pH within the range 5–7 (Liu et al., 2010, 2014). This explains the recurring observation that the N₂O/(N₂O + N₂) product ratio of denitrification increase with decreasing soil pH (Liu et al., 2010; Simek and Cooper, 2002; Cuhel et al., 2010).

We have previously shown that the intensification of plant production in Nepalese agriculture results in soil acidification, and that the soils acidified by intensive cultivation had higher N₂O/(N₂O + N₂) product ratios than soils under traditional cultivation. We hypothesized that this would imply higher N₂O emissions by intensification, exceeding that predicted by IPCC-assumption that fertilizer-induced N₂O–N emission amounts to ~1% of the fertilizer –N (IPCC, 2007).

This hypothesis needs to be tested rigorously in field experiments, however, since the N₂O emission from intact soil–plant system is controlled by numerous other variables such as temperature, moisture content, oxyanion concentrations and available organic carbon. In this study, we have measured the field fluxes of N₂O in traditional farming systems and adjacent fields of relatively similar soil types and had been intensified for more than 20 years prior to sampling, thus allowing pairwise comparisons as affected by intensification.

2. Materials and methods

The study area lies within a sub-watershed called as Ansikhola watershed of the central mid-hills of Nepal. The area has an annual rainfall of 1389 mm and average maximum and minimum temperature is 25 °C and 17 °C (Dahal et al., 2007). Two sites based on water management were selected with one site in Bari and another site in Khet. Bari is an area with rainfed upland leveled or sloping terraces and is permanently flooded. Khet is a lowland area with bunded and leveled terraces that is periodically flooded. Soil management in Khet include frequent flooding of the soils (for rice) for over 40 years. The cropping pattern in Bari and Khet are presented in Table 1. The Khet soils are finer textured than the Bari soils (Table 2). Within these two sites we studied adjacent plots with Intensified cropping system (IC) and more Traditional cropping system (TC) thus

allowing a pairwise comparison of soils from such contrasting plots to study the effects of intensification. The Intensified plots had a history of >20 years of intensive cultivation, i.e. high inputs of fertilizers and three crops per year. The Traditional plots had a history of lower N inputs and only two crops per year (Table 2). All farmers used compost based on farmyard manure, but the amounts of compost applied per year varied (Table 2).

2.1. Soil sampling

On each day of flux measurements, soil samples (0–15 cm depth) were taken in each plots (TC and IC) of both sites to determine soil mineral nitrogen (NH_4^+ and NO_3^-) content. The samples were immediately taken to laboratory and analyzed (within two days). Soil temperature and soil moisture content was also measured, twice on each day of flux measurements. The temperature was measured by inserting a temperature probe (Luster Leaf 1625 Digital Soil Thermometer). Soil moisture content was measured with a soil moisture meter (TDR 200 Field Scout, Spectrum Technologies Inc) inserted to a depth of 11.9 cm.

To determine the soil physical and chemical characteristics, four replicate soil samples (0–15 cm depth) were taken at random with each field. The soils were sieved (5 mm) to remove plant residues and coarse materials, and 200 g of each replicate soil samples were air dried and stored in plastic bags until analyzed for soil physical and chemical parameters. Soil samples for bulk density were also taken by pressing steel cylinders (101.42 cm³ volume) into the soils according to Blake and Hartge (1986).

2.2. Laboratory analyses

The soil samples for mineral nitrogen (NH_4^+ and NO_3^-) content were immediately taken to laboratory and analyzed. Bulk density (BD) was measured by drying intact soil cores taken by steel cylinders at 105 °C. Soil texture was determined by the hydrometer method. Soil pH was measured by dispersion of soil in 0.01 M KCl (0.2 g soil mL⁻¹).

2.3. Gas flux measurement

N₂O-emission was measured in both intensified and traditional agricultural plots (treatments) at upland (Bari) and lowland (Khet) sites (land use). Starting in first of June, intensive weekly measurements were applied throughout the rainy season (June to August), and changed to biweekly measurements during autumn (September to November), winter (December to February) and spring (March to May). The measurements were done over a period of one year from June 2009 to May 2010. The gas fluxes were measured using a closed chamber technique with four replicates within 1 × 1 m microplots at each treatment in each land use. The chamber has 220 mm internal diameter and 270 mm height. The cutting edges of the chamber were pressed into the soil up to 20 mm depth. The chambers were equipped with 12 mm diameter hole at the top of each chamber and were capped by butyl rubber stopper once the chamber was in place. Gas samples were taken transferring gas into 12 mL evacuated (up to 10⁻¹ kPa) vials. Gas samples were taken at zero and two hours after chamber deployment by a 20-ml disposable syringe and transferred to evacuated glass vials resulting in an overpressure. Gas samples were taken during afternoon between 14 and 15 h. Soil temperature and moisture were also measured during each time flux measurements.

Gas samples were shipped to the Norwegian University of Life Sciences for analysis using a Gas Chromatography equipped with electron capture detector (ECD) for N₂O analysis. The N₂O was analyzed as described by Sitaula et al. (1992).

$$F = (V/A)(\Delta C/\Delta t)$$

where F is the N₂O emission rate ($\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$), V is the volume of chamber above the soil (m³), A is the cross-section of chamber (m²), ΔC is the concentration difference between zero time and time t ($\mu\text{g N}_2\text{O-N m}^{-3}$), and Δt is the time duration between two sampling period (h).

2.4. Data analysis

All statistical analyses were performed by using software SAS (Institute Inc. Cary, NC, USA) and SPSS (version 19.0). The effect of N input on N₂O fluxes was analyzed by General Linear Model Procedure. The multiple comparison of means of fluxes in two landuses was carried out using Student–Newman–Keuls (SNK) test and LSD at $\alpha = 0.05$. Paired T-test was used for mean comparison of N₂O fluxes in TC and IC in both landuses. Linear regression was conducted where the dependent variable was N₂O fluxes and independent variables were soil moisture and temperature. The model incorporates the effects of moisture and temperature on the N₂O fluxes.

3. Results

3.1. Temporal variability of N₂O emissions in upland (Bari)

Fig. 1 illustrates the N₂O fluxes measured at the study site during the whole year in 2009 and 2010. Episodes of high N₂O emissions were observed following either rainfall events or fertilizer application. The results showed a clear seasonality

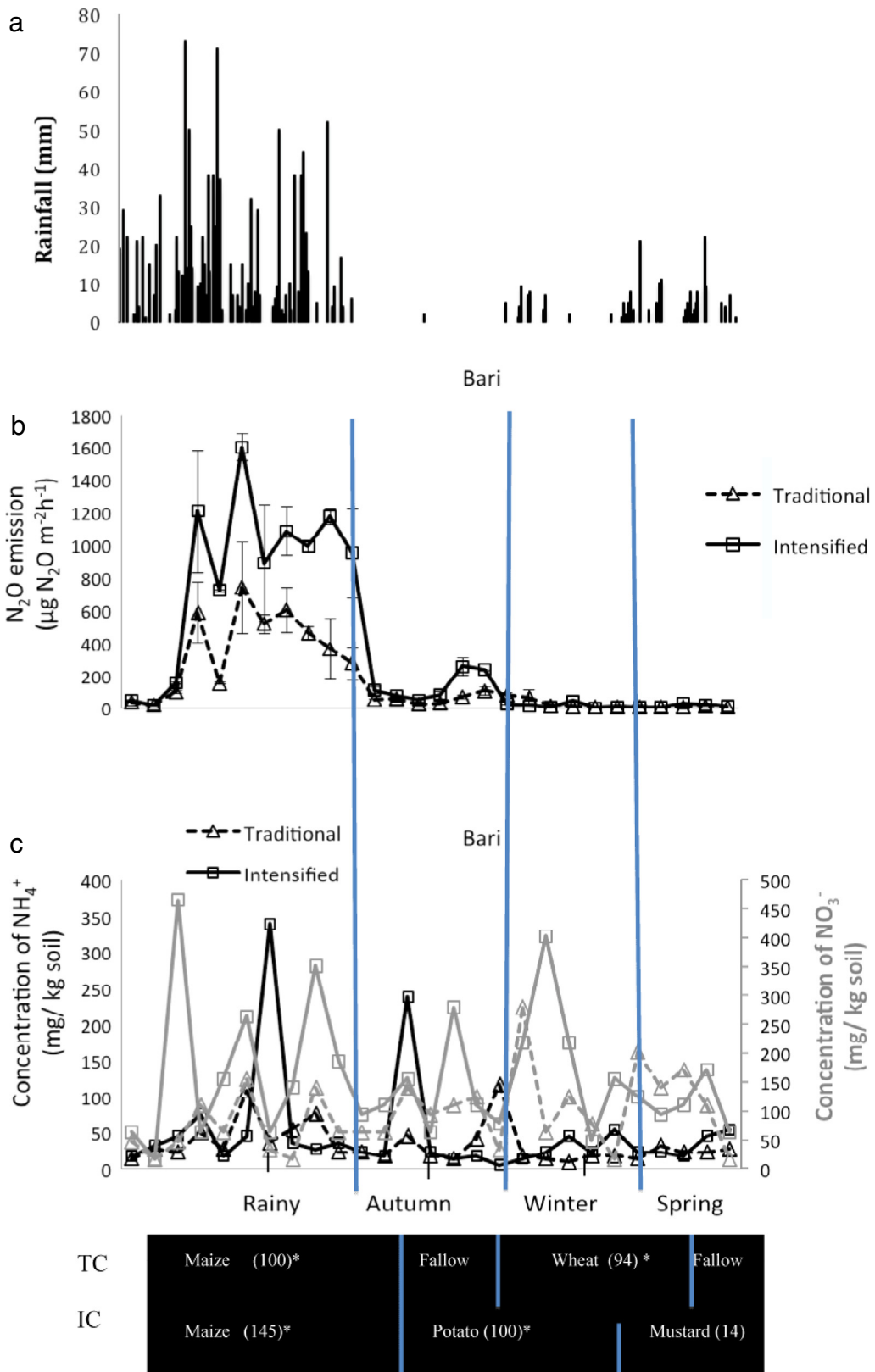


Fig. 1. Seasonal dynamics of (a) daily precipitation (b) N_2O emission (mean \pm SE, $n = 4$) and (c) NH_4^+ and NO_3^- in IC (—) and TC (---) of permanently drained soils in Bari lands. All four figures have same description of horizontal axis.

Table 3

Effect of crop intensification on N₂O fluxes for the whole measurement period at Upland (*Bari*) and Lowland (*Khet*).

Site	N ₂ O ($\mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$)		N ₂ O ($\mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$)	
	<i>Bari</i>		<i>Khet</i>	
	Mean	Range	Mean	Range
Traditional agricultural system	157.68 ^a	0.50–1231	32.49 ^a	0.13–193
Intensified agricultural system	351.44 ^b	0.43–2017	115.12 ^b	0.05–387

a & b Means followed by different lower case are significantly different at $p < 0.001$.

with highest emission rates in the rainy season (Fig. 1). The highest peaks appeared after the application of fertilizer in maize cultivation in both TC and IC. Peaks appeared for IC immediately after the addition of N fertilizer for potato. The episodic peak also appeared for TC during the fallow period and start of the wheat cultivation season. The comparison of means showed that N₂O emission ($\mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) in IC was significantly higher than in TC ($p < 0.001$). The range of N₂O emission rates in *Bari* land varied from 0.5 to 1231 and 0.4 to 2017 $\mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$ in TC and IC, respectively (Table 3). N₂O emissions during the rainy season contributed most to the measured N₂O flux, both in TC and IC (Fig. 3).

3.2. Temporal variability of N₂O emissions in lowland (*Khet*)

As for *Bari*, the N₂O fluxes showed a clear seasonality with highest emission rates in the rainy season (Fig. 2). The range of N₂O emission rates in *Khet* land varied from 0.13 to 193 and 0.05 to 387 $\mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$ in TC and IC, respectively (Table 3). The comparison of means showed that N₂O emission ($\mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) in IC was significantly higher than in TC ($p < 0.001$). The peaks were generally higher during the rainy season compared to other seasons. The peak spiked for N₂O emission during winter in IC as a result of N input for potato cultivation. However, the N₂O emission was significantly higher for *Bari* land compared to *Khet* land ($p < 0.05$).

3.3. Environmental factors affecting N₂O emissions

Soil temperature, precipitation and soil moisture were monitored to serve the analysis of N₂O emissions from the study sites. Fig. 4(a)–(b) show the measurements of soil temperature and moisture each time gas sampling in both *Bari* and *Khet* lands. The accumulated rainfall was 1482 mm over the study period. The measured soil moisture content in *Bari* ranged from 5% to 36% in TC and 4%–33% in IC and *Khet* land ranged from 11% to 38% in TC and 15%–52% in IC, over the study period. In *Bari*, the soil moisture was periodically the rain to IC and TC whereas in *Khet*, IC had systematically higher soil moisture than TC. Variations in the soil moisture were mainly driven by precipitation events.

N₂O flux was positively correlated with soil moisture content (%) in both treatments and landuses. However, the significant correlation existed between N₂O flux and moisture content in intensified agricultural system of periodically flooded *Khet* lands ($p < 0.001$). Furthermore, N₂O flux was positively correlated with soil temperature and the correlation is significant in intensified agricultural system of periodically flooded *Khet* lands ($p < 0.05$).

3.4. Soil ammonium and nitrate contents

Soil ammonium and nitrate are the major substrate of N₂O production. Therefore soil ammonium and nitrate were monitored in the treatments in both landuses at each sampling date during the sampling period. Figs. 1 and 2 presents the measured soil NH₄⁺ and NO₃⁻ contents. Soil NH₄⁺ and NO₃⁻ peaks were observed in both landuses following the top-dressing of N fertilizers. The concentrations were generally higher in intensified agricultural system. In the IC of *Khet* land, the release of the initial N fertilizers (especially urea and DAP) at the rate of 144 kg N ha⁻¹ during rice plantation and 160 kg N ha⁻¹ during maize planting stage, increased the concentration of NH₄⁺ and NO₃⁻ until end of rainy season for rice plantation and until mid autumn to end of winter for potato plantation. Likewise, in the IC of *Bari* land, the release of applied N fertilizers at the rate of 145 kg N ha⁻¹ during maize plantation, 100 kg N ha⁻¹ during potato plantation and 14 kg N ha⁻¹ during mustard planting stage, increased the concentration of NH₄⁺ up to 339 mg/kg of soil and NO₃⁻ up to 383 mg/kg of soil. For the TC where N application is less compared to IC, the soil NH₄⁺ and NO₃⁻ concentrations remained at a low level throughout. In this study, the high N₂O fluxes were observed following fertilizer N application, indicating that the variations of daily N₂O fluxes were closely related to soil mineral N availability during the study periods.

3.5. N₂O emission factors for intensive cultivation

Most of the N₂O emission took place during the rainy season. For *Bari*, the accumulated N₂O emissions for the whole year were 15.41 and 7.23 kg N₂O/ha for IC and TC respectively (Fig. 3a). Based on the available data on nitrogen input, we calculated an emission factor for the transition from TC to IC (EF); i.e. $\Delta\text{N}_2\text{O-N}/\Delta\text{N}_{\text{fertilizer}}$, where $\Delta\text{N}_2\text{O-N}$ is the cumulated

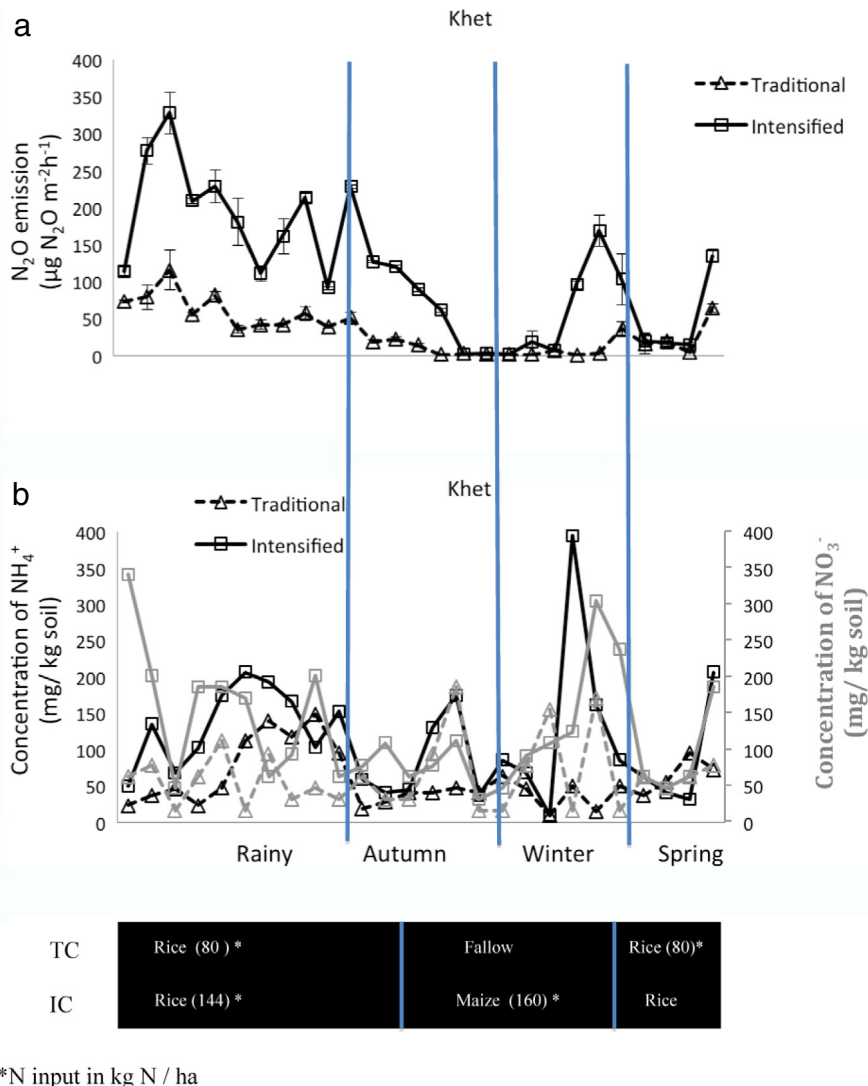


Fig. 2. Seasonal dynamics of (a) N_2O emission (mean \pm SE, $n = 4$) and (b) NH_4^+ and NO_3^- in IC (—) and TC (---) of periodically drained soils in *Khet* lands. All four figures have same description of horizontal axis.

emission in IC minus that in TC, $\Delta N_{\text{fertilizer}}$ is the annual N input to IC minus that in TC. Such emission factors were also calculated for the individual seasons. Thus $\Delta N_{\text{fertilizer}}$ is cumulated N_2O emission for that season and $\Delta N_{\text{fertilizer}}$ is N input to IC for that season minus that to TC. The EF for the whole year was 0.08 for the permanently drained soils in *Bari* land. The emission factor for rainy season (EF_{RB}) was 0.11; EF for autumn and winter (EF_{AWB}) was 0.03 and for spring season (EF_{SB}) it was 0.003.

The annual accumulated N_2O emissions in periodically flooded soils (*Khet*) were 5.43 and 1.46 kg N_2O /ha for IC and TC respectively (Fig. 3b). We calculated emission factor (EF) also for *Khet* for the whole year as well as for the individual seasons. The EF for the whole year was 0.02 for periodically flooded soils in *Khet* land. The emission factor for rainy season (EF_{RK}) was 0.02, EF for autumn and winter (EF_{AWK}) was 0.01 and for spring season (EF_{SK}) it was 0.002.

3.6. Emission intensity

Based on measured rice yields in both TC and IC in *Khet* lands (2009) we calculated “ N_2O intensity”. The measured rice yields were 2.75 t ha $^{-1}$ and 3.04 t ha $^{-1}$ in TC and IC, respectively. While the N_2O emission was also higher for IC compared to TC. Together, this resulted in higher N_2O intensity calculated as g N_2O per kg of rice production in intensively cultivated land. The N_2O intensity were 1.08 g N_2O per kg and 0.37 g N_2O per kg of rice production, in IC and TC, respectively, which is 2.9 times higher for IC than for TC.

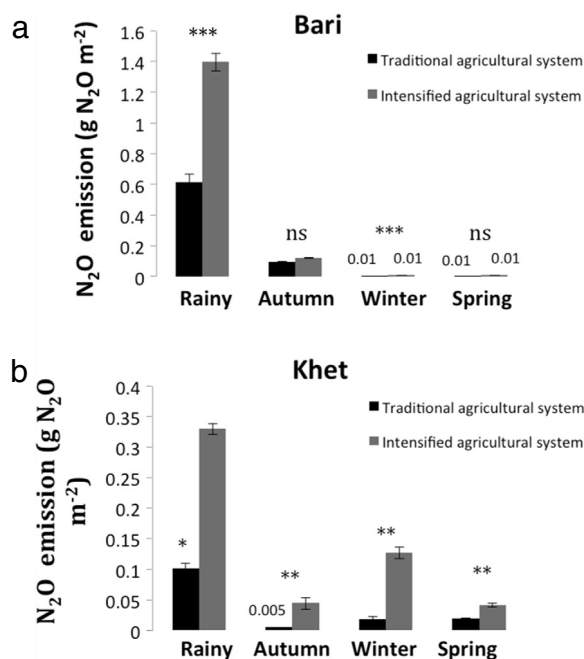


Fig. 3. Seasonal accumulated fluxes of N₂O (a) *Bari* land and (b) *Khet* land. *, ** and *** are significantly different at $p < 0.05$, 0.01 and 0.001 respectively.

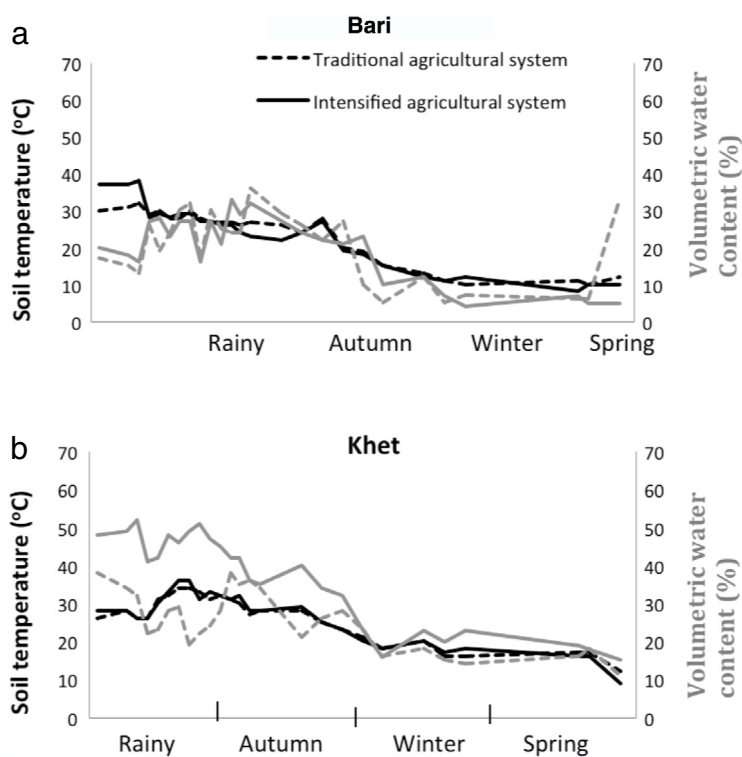


Fig. 4. Soil temperature and moisture (%) in (a) *Bari* land and (b) *Khet* land.

4. Discussion

Since the Green Revolution in the 1960s, synthetic fertilizers have played a key role in increasing agricultural production in Nepal. Urea is a preferred N fertilizer due to low price, high N content and availability on the market, most farmers prefer to use urea rather than any other fertilizers. Due to a reduction of animal production, the amounts of farmyard manure (FYM)

have decreased (Raut et al., 2011). Moreover, farmers still believe that high-yielding crop varieties require the use of greater amounts of chemical fertilizer if production is to be increased. The poor extension services and lack of awareness among farming communities is leading to a disproportionate use of Nitrogen–Phosphorus–Potassium (NPK) fertilizers that may cause nutrient imbalances in the soil. However, crops usually use the N fertilizers with low efficiency (Cassman et al., 2002; Tilman, 2001) and the surplus N in soils is characterized as one of the most important factors stimulating N_2O emissions from crop fields (Bouwman, 1996; Bouwman et al., 2002; Davidson and Verchot, 2000). Our recent study in the same study sites (Raut et al., 2012) demonstrated that intensive cropping systems invariably lowered the soil pH. This had increased the $\text{N}_2\text{O}/(\text{N}_2 + \text{N}_2\text{O})$ product ratio of denitrification during standardized anoxic incubations (Raut et al., 2012). This phenomenon has been ascribed to a negative effect of low pH on the organisms' ability to produce functional N_2O reductase Liu et al. (2010).

The ongoing intensification of cropping systems will thus result in increased emissions of N_2O and the prospects are potentially worse than those predicted by the increasing doses of N-fertilizers. As hypothesized that this would lead to high N_2O emission factors (EF), and tested this by measuring N_2O emissions from fields on which intensified crop production (IC) had been practiced for the last 20 years, and adjacent fields with more traditional crop production (TC). The high EF appeared to confirm our hypothesis that intensification will lead to higher emission of N_2O than that predicted by the increase in nitrogen inputs, and that this is due to the soil acidification. Direct soil N_2O emissions from agriculture are often estimated using the default IPCC emission factor (EF) of 1%. These factors are 2–8 times higher than the EF values used by IPCC to calculate emission as a function of fertilizer level. However, our results showed the EF value was 8 and 2 times higher in permanently drained soil and periodically drained soils respectively, than the EF values used by IPCC. As the EF calculated for the seasons where fertilizer N is applied, the EF for rainy season was 11% for permanently drained soils, which is higher than EF for rainy seasons in periodically drained soil. In permanently drained soils in Bari land, the result showed the emission factor for rainy season (EF_{RB}) was 0.11, for autumn and winter (EF_{AWB}) was 0.03 and for spring season (EF_{SB}) was 0.003. However, a large variation in EFs exists due to differences in environmental factors, cropping intensity and management (Lesschen et al., 2011).

For various reasons, the emission of N_2O from a soil is not necessarily proportional with the $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$ product ratio as determined in our previous study in the same study area (Raut et al., 2012). As illustrated in that study, the accumulation of N_2O is normally a transient phenomenon, and the cumulated N_2O is reduced; at least after depletion of NO_3^- and NO_2^- . This is probably similar to what happens if soils are flooded for long periods: long lasting anoxic conditions and marginal transport of N_2O from the system due to water logging. This would explain why N_2O emissions are generally low from periodically flooded Khet lands as compared to permanently flooded Bari lands. The same was also explained by Tsuruta et al. (1997) where they found lower N_2O emission in rice fields, and the main product of denitrification in such systems appear to be N_2 (Mosier et al., 1989). For permanently drained soils, on the other hand, it appears that the emissions of N_2O from this study are in proportion to the $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$ product ratio as measured with same soil in Raut et al. (2012).

N_2O fluxes were positively correlated with soil temperature and the correlation is significant in intensified agricultural system of periodically flooded Khet lands ($p < 0.05$). The positive correlation is consistent with other findings by Papen and Butterbach-Bahl (1999), and Yuping et al. (2008). N_2O flux was positively correlated with soil moisture content (%), which is in good agreement with other studies (Lemke et al., 1998; Rafique et al., 2011; Zhu et al., 2013a). Greater N_2O emissions during rainy season at both landuses were associated with higher soil moisture content that enhance microbial activity. The high soil moisture during rainy season, likely result in respiration rates exceeding the diffusion rate of O_2 into the soil, thus causing partly anoxic conditions. This would suggest that denitrification gains importance stimulating N_2O production during rains, presumably explaining the increase in emission rates (Zhu et al., 2013b).

The N inputs during rainy season, on the other hand, enhances the microbial activity. Substrate limitation of microbial metabolism was also reflected by the results of ex situ incubations of soils from the same soils (Raut et al., 2012). Denitrification was generally constant during Phase DEN (Phase without substrate addition) despite ample supply of NO_3^- (by washing the soil with KNO_3 solution prior to incubation), while denitrification immediately increased after glutamic acid addition in Phase SIDEN (Phase with substrate addition). Whereas Phase DEN probably reflects the pool of denitrifiers with an existing denitrification proteome (Liu et al., 2010), Phase SIDEN reflects the pool of denitrifiers which can be induced by addition of readily available carbon (Raut et al., 2012). Our study together with our incubation results suggest severe C limitation of denitrifiers and may thus explain the lower N_2O emissions during spring compared to rainy season and other seasons where the N input is high.

In conclusion, the study provides compelling evidence that intensification of agriculture has enhanced the higher N_2O emission as quantified by field flux measurement and our previous study on product stoichiometry of denitrification in standardized anoxic incubations. Thus, this implies that intensification enhance N_2O emission than that predicted by the IPCC (IPCC, 2006).

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.gecco.2015.06.004>.

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